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Original Article

COMPARISON OF ANTIBACTERIAL ACTIVITY OF LEAVES EXTRACTS OF *TECTONA GRANDIS, MANGIFERA INDICA,* AND *ANACARDIUM OCCIDENTALE*

KRISHNANANDA KAMATH K.*, A. RAMAKRISHNA SHABARAYA

Department of Pharmaceutics, Srinivas College of Pharmacy, Valachil, Mangalore 574143, Karnataka, India
Email: kamathkrishnananda@yahoo.com

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ABSTRACT

Objective: World Health Organization estimated that 80% of people worldwide rely upon herbal medicines for some aspect of their primary healthcare. For most of the herbs, the specific ingredient that causes therapeutic effect is not known. Bacterial infections are one of the prominent causes of health problems, physical disabilities and mortalities around the world. Plants have been used in medicine as antimicrobial agents since ancient times could provide a promising solution for drug-resistant species. The present study involves comparison of the antibacterial activity of ethanolic extract leaves of *Tectona grandis* (teak), *Mangifera indica* (mango), and, *Anacardium Occidentale (cashew).*

Methods: Authentication, morphological studies and phytochemical screening studies on alcoholic extracts of leaves were carried out. Antibacterial activity was carried out by using different concentrations of extracts on bacterial strains of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* by using agar well diffusion method and results were compared.

Results: Preliminary phytochemical screening of extracts revealed the presence of alkaloids, glycosides, saponins, resins, tannins and flavonoids. Antibacterial activity was observed in the concentration range of 25-100 mg/ml for all the extracts except *T. grandis* leaves. They are effective at 50-100 mg/ml concentration.

Conclusion: Comparison of results reveals that leaves of *T. Grandis* have less antibacterial activity compared to *M. Indica* and *A. Occidantale* extracts.

Keywords: Teak, Mango, Cashew Leaves Extracts, Phytochemical Screening, Antibacterial activity

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INTRODUCTION

Herbal medicine has become an item of global importance both as medicinal and economical. The use of plant and plant products for therapeutic use is known since time immemorial. Herbs are used to treat various infectious diseases worldwide. They are most effective, cheaper and alternative sources of drugs. Plants play a vital role in curing various ailments and herbal remedies are getting increasing patient compliance as they are devoid of typical side effects of allopathic medicines. The effective plant constituents can combat human and plant pathogenic bacteria, fungi and virus without any side effects and environmental hazards. Due to this favourable reason, search for plant products with antimicrobial properties intensified in recent years [1-3]. Although usage of these herbal medicines has increased, their quality, safety and efficiency are serious concerns in industrialised and developing countries.

Bacterial infections are one of the prominent causes of health problems, physical disabilities and mortalities around the world. Plants that have been used in medicine as antimicrobial agents since ancient times could provide a promising solution for drug-resistant species. The natural products are found to be more effective with least side effects as compared to commercial antibiotics this is why they are used as an alternate remedy for the treatment of various infections. The most essential of bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Bioactive constituents have found applications as naturally occurring antimicrobial agents in the field of preservation, pharmaceutics, phytopathology, etc. Increasing failure of chemotherapeutics and the resistance exhibited by pathogenic microbial infectious agents against antibiotics have led to the screening of medicinal plants for their potential antimicrobial activities [4, 5].

There are several reports regarding the antimicrobial activity of crude extracts prepared from plants. Some of the active principles of the bioactive compounds are preferred for their therapeutic purposes either as a single entity or in combination, so as to inhibit the life processes of microbes [6-7]. Considering above facts present research work was aimed at comparison of the antibacterial activity of leaves extract containing *Tectona grandis Linn.* Family *Verbenaceae,* extracts of *Mangifera indica (L.),* Family *Anacardiaceae,* and *Anacardium occidentale* Linn. Family *Anacardiaceae.* Plants were investigated for antibacterial activity against *S. aureus, E. coli* and *P. aeruginosa*.

MATERIALS AND METHODS

Materials

Gentamycin Sulphate USP gift sample procured from Ranbaxy Laboratories Ltd., Madkaim, Ponda, Goa. Microbiological media, Mueller Hinton Agar (MHA) (Himedia) was procured from the department of microbiology, Srinivas College of pharmacy, Mangalore. All the other chemicals used in the formulations were of analytical grade.

Methods

Collection, identification and extraction

Fresh leaves of *T. grandis, M. indica and A. Occidentale* were collected from the coastal region of Udupi district, Karnataka, India in the month of February 2015. The plant leaves were authenticated by Prof. Gopal Krishna Bhat, Department of Botany, Poornaprajna College, Udupi, Karnataka, India. The leaves were washed 2-3 times with running water, air dried under shade and made into coarse powder by using a grinder, stored in airtight containers in cool place.

Soxhlet extraction of dried leaves

Collected leaves were air-dried, powdered and subjected to soxhlet extraction. Approximately 50 to 60 g of the powder was extracted with ethyl alcohol. Evaporation of the solvents from the extracts was done by using rotary vacuum evaporator at 35-50°C. A sticky mass was obtained after evaporation of extracts, labelled and stored at 2-8°C. The percentage yields were calculated [8].

Table 1: Parameters used for extraction from dried leaves

Parameters	*T. grandis*	*M. indica*	*A. occidenatle*
Dried powder taken	55 g	58 g	55 g
Amt. Ethanol taken	350 ml	350 ml	350 ml
Time taken for extraction	5 h	5 h	5 h
RPM of rotary evaporator	40-45 RPM	35-40 RPM	35-40 RPM
Temp. of water bath	35-45°C	40-50°C	40-50°C
Wt. of extract.	9.54g	11.68 g	10.48 g
%Yield (w/w)	17.34%	20.13%	19.05%
Alcoholic extract Color	Dark red color	Greenish Yellow color	Greenish color

Preliminary phytochemical screening

Extracts from the leaves were subjected to qualitative analysis (table 2) to check the presence alkaloids, carbohydrates, glycosides, saponins, phytosterols, fixed oils and fats, resins, phenols, tannins, flavonoids, proteins, amino acids, and triterpenoids [8-10, 13].

***In vitro* antibacterial activity of extracts of plants**

Ethanol extracts of *T. grandis, M. indica* and *A. occidentale* were dissolved in a few drops of Dimethylsulfoxide (DMSO) and made up with distilled water to give a stock solution of 100 mg/ml separately. From this stock solution 25, 50 and 75 mg/ml concentrations were prepared. The stock solutions were kept at 4-8°C. Standard bacterial organisms from the ATCC were obtained from the department of microbiology, Srinivas College of pharmacy, Mangalore. *S. aureus* (ATCC25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used. The organisms were first isolated on nutrient broth for 24 h and then diluted to 1:1000 with the sterile nutrient dextrose broth. The dilutions formed were used as bacterial stock solutions for the agar-well diffusion assays.

Preparation of media, Direct sensitivity testing

Mueller Hinton agar media was used. The media was prepared by adding 11.40 g of agar powder to 300 ml of distilled water and the mixture was boiled. The solution was autoclaved at 121°C for 15 min and cooled to 50°C in a water bath. It was then transferred into sterile plates. It was allowed to cool and solidify under sterile conditions and then incubated for 24 h at 37°C to ensure that there is no bacterial contamination. Wells of 6 mm diameter and 5 mm depth were made in the solidified agar using a sterile borer.

Agar-well diffusion assay

Cultures of *E. coli*, *S. aureus* and *P. aeruginosa* were inoculated separately onto agar before solidifying. Then it was transferred to each Petri dish. Prepared 1 ml extracts of concentration 25, 50, 75, 100 mg/ml of the test and 10 µg/ml of Gentamycin Sulphate USP (positive control) were dispensed into the wells. The plates were incubated at 37°C for 24 h. The sensitivity of the test organisms to the all the three above extracts was determined by measuring the diameters of the zone of inhibition surrounding the wells [11, 12].

RESULTS AND DISCUSSION

Morphology of leaves was studied and complies with the specifications. Plant leaves are depicted in fig. 1, 2 and 3.

***In vitro* antibacterial activity of ethanolic extracts of plants**

The extracts were studied for antibacterial activities of using agar well diffusion method; results are depicted in table 3. The selected test organisms used in this study are associated with various forms of human infections. These organisms are common causes of infections in a patient with burns and other types of wounds. Gentamicin sulphate USP, used as the positive control, showed sensitivity to test organisms with 25-29 mm of zone diameter showed a maximum inhibitory effect compared to leaves extracts. Ethanol extract of *T. Grandis* with concentration 25 mg/ml did not show any effect on the growth of microorganisms, Only 50, 75 and 100 mg/ml concentrations were effective.

Fig. 1: Leaves of *T. Grandis*

Fig. 2: Leaves of *M. indica*

Fig. 3: Leaves of A*. Occidantale*

Preliminary phytochemical screening of extracts was done and revealed the presence of alkaloids, glycosides, saponins, resins, tannins, phenols and flavonoids as represented in table 2.

In the case of *M. Indica* and *A. Occidentale,* all test concentrations were effective. Extract of *A. Occidentale* gave maximum zones of inhibition for 25, 50, 75 and 100 mg/ml concentration respectively (table 3) when compared to other extracts.

The antibacterial activity could be due to different classes of compounds present in extracts. Some of the classes of compounds identified in the crude extract were mainly alkaloids, phenols, flavonoids, and tannins have been reported to possess antibacterial activity [13-16]. Further fractionation of the extracts could yield fractions or compounds with higher antibacterial activity. The antibacterial activity of herbal plants is may be due to adsorption of polyphenols to bacterial membranes with disruption of the membrane and subsequent leakage of cellular contents. In this study, ethanolic *A. Occidentale* leaves contains the phenolic active compound, such as anacardic acid which shows antimicrobial effect [17-21].

Table 2: Phytochemical Screening of extracts of *T. Grandis, M. indica* and *A. Occidentale*

Chemical constituents	Tests	*T. Grandis*	*M. indica*	*A. Occidentale*
Alkaloids	1. Mayer's test	+	+	+
	2. Dragendroffs test	+	+	+
	3. Wagners test	+	+	+
	4. Hagers test	+	+	+
Carbohydrates	1. Molischs Test	-	+	+
	2. Bendicts test	-	+	+
	3. Fehlings test	-	+	+
	4. Barfoed's Test	-	+	+
Glycosides	1. Brontagers test	-	+	_
	2. Legal Test	-	+	_
Saponionis	1. Foam test	+	-	+
	2. Froth Test	+	_	+
Phytosterols	1. Salkowski test	-	_	+
	2. Leibermann Burchard test	-	_	+
Fat and Oils	1. Stain test	-	_	+
Resins	1. Acetone and water test	+	+	+
Phenols	1. Ferric Chloride test	-	+	+
Tannins	1. Alkaline reagent test	-	+	+
	2. Gelatin test	-	+	+
Flavonoids test	1. Lead Acetate test	+	+	+
	2. Shinoda test	+	+	+
	3. Zn-HCl reduction test	+	+	+
	4. Alkaline reagent test	+	+	+

Table 3: Antibacterial activity of leaves extracts of *T. Grandis M. Indica A. Occidentale* Diameter of zone inhibition (mm)

Leaves extract conc. (mg/ml)	*S. aureus*			*E. coli*			*P. aeruginosa*		
	T Grandis	*M. indica*	*A. occidentale*	*T Grandis*	*M. indica*	*A. occidentale*	*T Grandis*	*M. indica*	*A. occidentale*
25	Nil	6-8	6-9	Nil	5-8	6-9	Nil	5-7	7-10
50	6-8	8-12	11-14	6-9	9-11	10-12	7-9	10-12	11-13
75	7-9	13-15	16-19	9-10	11-14	16-18	8-10	14-17	15-18
100	10-13	17-20	22-28	12-15	15-17	21-24	13-16	18-19	22-26
Gentamycin (10 µg/ml)	24-27	22-26	24-29	23-27	23-25	24-28	22-26	25-27	26-29

Flavonoids have been recognised as having a protective effect in plants against microbial invasion by plant pathogens. Flavonoids have been shown to possess important biological activities, including antifungal and antibacterial activities. This study reports that the leaves of *M. indica* contain alkaloids, anthracenosides, coumarins, flavonones, sugars, tannins, steroids and saponins. Some of these compounds have been reported to possess antimicrobial activity. Flavonoids from mango leave possess antibacterial, antifungal activities against pathogenic fungi. Since flavonoids are available at low cost and are less toxic to humans, they are highly suitable for treating such human diseases. The demonstration of activity against both Gram-negative and Gram-positive bacteria is an indication that the plant can be a source of bioactive substances that could be a broad spectrum of activity [22-29].

In conclusion, the results of the present study revealed that selected plants possess antibacterial activity. According to this study, ethanol extracts produced zone of inhibition of 6-26 mm against microorganisms. All the leaf extracts showed an antibacterial activity compared with the gentamycin sulphate positive control against the study organisms.

CONCLUSION

From the findings it was revealed that all the three leaves extracts of *T. Grandis, M. Indica, and A. Occidantale* contain bioactive compounds that are effective against the test organisms, thus the crude extracts can be used for the treatment of infections caused by the organisms. Another advantage of using these herbs as an antimicrobial agent is that there is no harmful effect on the body and there is less chance of development of resistance in bacteria against these herbs. Further studies involve the formulation of semisolid dosage forms containing individual and mixture of all the three extracts. So, herbal remedies can be recommended in various medical treatments for the cure of different disease.

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CONFLICT OF INTERESTS

Declared none

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